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LabLink

Laboratory Information from the Michigan Department of Community Health -- Bureau of Laboratories

Vol. 4 No. 3 Winter 1999

Martin Leaves Michigan for CDC

Barbara Robinson-Dunn, Ph.D. and Susan L. Shiflett

Robert Martin, Dr.P.H., laboratory director at the Michigan Department of Community Health (MDCH), left on January 29, 1999 to accept a position at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia. At the CDC, he will be the director of the division of laboratory systems in the Public Health Practice Program Office (PHPPO).

Martin had an illustrious career while at MDCH. Following graduation from Michigan State University in medical technology, and an internship at Pontiac General Hospital, he accepted a position with the Western Michigan Division of the Michigan Department of Public Health as a microbiologist. In 1976, Martin obtained his master of science degree from Michigan Technological University (MTU) while working part time in the Upper Peninsula Laboratory in Houghton. Desiring additional education, he obtained both a master of public health and a doctor of public health degree from the University of North Carolina.

Martin moved his family back to Houghton in 1979 to become the laboratory director and chief of Upper Peninsula Divisions. While there, he directed laboratories in Houghton and Powers and taught medical microbiology and epidemiology at MTU. In 1982, he accepted a position with MDPH in Lansing as deputy chief of the microbiology division and became the division chief the following year. The laboratory environment and testing practices began to change at that time.

Realizing the role that public health laboratories must play to maintain a leadership position in the laboratory community, Martin sought to incorporate new, more specific testing methodologies while maintaining necessary conventional techniques. This included establishment of the molecular biology section which is now recognized both nationally and internationally, expansion of the newborn screening program that ensures all Michigan newborns are examined for seven metabolic or genetic disorders, development of a regional public health laboratory system in Michigan that utilizes county health department laboratories to provide services best done in a decentralized environment and establishment of the MDPH laboratories as a site for evaluation of new methodologies. He also successfully campaigned to discontinue services at the state laboratory that most hospital laboratories could easily provide.

One of his strong points was opening the lines of communication both within the laboratory and with our public health and laboratory partners. (*LabLink* is an example of his open communication policy.) Martin maintained an open door policy in his office where there were often lively discussions on sports, mountain climbing and everyday life as well as laboratory issues.

Martin published extensively and he also encouraged his colleagues to publish. He was the recipient of numerous awards including the College of Natural Science Outstanding Alumni Award from Michigan State University. He helped to educate future medical technologists by teaching medical microbiology at MSU.

Martin was active in many professional organizations while at MDCH. As a member and president of the Association of Public Health Laboratories (APHL), he was influential in establishing national guidelines for the laboratory diagnosis of Lyme Disease, instituting rapid testing for *Mycobacterium tuberculosis*, initiating a national approach to *Salmonella* serotyping and launching national regional public health laboratory testing in cooperation with CDC. He traveled to India as part of the World AIDS Foundation Project to help establish standardized HIV testing. He is also active in the American Society for Microbiology, currently heading the Public Health Division, the American Public Health Association, and locally, the Michigan Public Health Association.

He will be greatly missed in Michigan as both a colleague and a friend. His wonderful talents that served to benefit the citizens of Michigan will now be serving the national and international laboratory community. We wish him well knowing that we will continue to work with him in a much larger context.

Mycobacterium bovis in Free-Ranging Michigan Deer

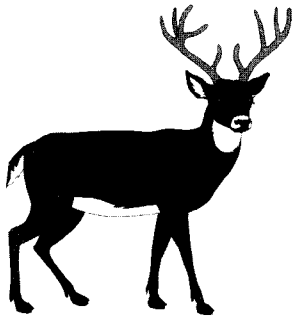
Barbara Robinson-Dunn, Ph.D.
Director of Microbiology

Bovine tuberculosis is a bacterial disease affecting the respiratory system primarily of animals, traditionally cows. Although all mammals can be infected with *Mycobacterium bovis,* this bacterium is rarely recovered from humans in industrialized countries. In 1975, a nine-year-old female white-tailed deer from Alcona County and in 1994 a four-year-old male deer from Alpena County were submitted to the Rose Lake Wildlife Disease Laboratory, Department of Natural Resources with lesions consistent with those produced by *M. bovis*. These findings were confirmed by the isolation and identification of *M. bovis* from autopsy specimens.

To assess the extent of the bovine tuberculosis problem in deer, an intensive effort has been made to check deer taken during the fall hunting season in the area surrounding the initial isolates. Over four years (1995-1998) a total of 17,157 deer have been tested with a small portion culture confirmed in the laboratories at MDCH and the National Veterinary Services Laboratory in Ames, Iowa. *M. bovis* has been identified in 228 of those deer. (Culture confirmation is in progress on 45 isolates at this time.) These studies have shown that the disease occurs in the free-ranging deer population in the five-county area of Alpena, Alcona, Montmorency, Oscoda and Presque Isle. *M. bovis* has not been identified in deer taken outside this area. *M. bovis* has also been isolated and identified in carnivores within the five-county area. These animals (five coyotes, two racoons and a black bear) most likely acquired their disease by consuming infected offal left following field dressing.

M. bovis has been identified in three separate herds of cattle in the-five county area. The molecular fingerprint of the cattle isolates matches those of the deer isolates. Because of the infected cattle, Michigan has received split state status as designated by the U.S. Department of Agriculture. Thus, cattle and goats designated to be shipped out of the quarantine area (bordered by I-75 and M-55) must have had a negative TB test within the last 60 days. This is not required of livestock from other areas of the state. Even so, some states are refusing to allow livestock from Michigan within their boundaries. The extent of the economic impact of these events has not yet been determined.

To eliminate *M. bovis* from Michigan livestock, it is necessary to reduce the number of deer in northeast Michigan. A number of measures are in place to achieve that goal. These include a ban on feeding deer, a ban on baiting deer after January 3, 1999, an increase in the number of deer taken by hunting and an almost unlimited increase in the number of antlerless permits available in the five-county area. The goal is to have less than 1% of the deer infected by the year 2003 with total elimination shortly thereafter. With the cooperation of all involved, it can and will happen.



(For further information on the bovine tuberculosis problem in Michigan, visit the DNR website:

http://www.dnr.state.mi.us/wildlife/division/roselake)

MICHIGAN TRAVELS TO JAMAICA

Dale Berry and Angie Schooley
Mycobacteriology/Mycology Unit

Two microbiologists from MDCH traveled to Kingston, Jamaica on November 9,1998 to visit Jamaica's National Public Health Laboratory (NPHL). In 1996, MDCH was chosen by the Association of Public Health Laboratories (APHL) to be a sister laboratory to NPHL. The trip was made possible through the joint efforts of APHL, CDC, the Pan American Health Organization (PAHO) and the Caribbean Epidemiology Center (CAREC). The goals of the visit were two-fold; first to assist NPHL with the implementation of the Bactec 460 radiometric broth isolation and susceptibility system for *Mycobacterium tuberculosis* and second to provide a workshop on acid-fast microscopy to laboratories in Jamaica and neighboring Caribbean countries.

Two weeks were spent in the mycobacteria section of NPHL focusing on the implementation of the Bactec 460 system. Prior to the training visit, NPHL had been performing only direct acid-fast microscopy and NaOH specimen decontamination. It was necessary to initiate NALC-NaOH digestion and concentration by centrifugation into their testing protocol. These methods would significantly improve the sensitivity of smear and culture detection of acid-fast bacilli. Implementation of the rapid Bactec testing method would provide reduced turn around time for culture detection of patients with TB and the subsequent antibiotic susceptibility.

Instituting newer technologies can present many challenges. Due to import restrictions, many chemicals, reagents and equipment necessary for rapid TB testing can be difficult to obtain in Jamaica. Delivery of the Bactec 460, which employs the use of a radioactive carbon substrate in the 12B broth, was delayed for about two years due to funding restraints and concerns regarding radioactive substances. Cyanogen bromide, commonly known as tear gas, and required for the niacin TB identification test, is prohibited from being imported. Acid-fast cording and antibiotic susceptibility patterns are the methods currently used for identification of *M. tuberculosis* at NPHL. However, NPHL is in the process of purchasing Bactec's NAP vials to be used as an additional TB identification test.

In addition to completing the implementation of the Bactec system, the second week was spent preparing and presenting a two-day wet workshop on acid-fast microscopy. Nineteen participants came from Jamaica, the Bahamas, Curacao, Bermuda, Barbados and the British Virgin Islands. The workshop focused on the use of the microscope, direct smear preparation, acid-fast staining methods and cross-contamination in laboratory testing. Due to limited appropriate laboratory facilities, direct microscopic examination for acid-fast bacilli is the standard testing protocol. Workshop attendees only performed direct microscopy in their laboratories, sending specimens to a central reference laboratory for culture testing.

Although many obstacles had to be overcome, the goals were accomplished by the end of the visit. Our Jamaican hosts were exemplary in extending their friendship, hospitality and assistance. We will continue our commitment to assist in the improvement of their tuberculosis laboratory testing services.

INFLUENZA UPDATE

Victoria Vavricka
Viral Serology/Viral Isolation Unit

In choosing the particular strains for the 1998-99 trivalent influenza vaccine, the Food and Drug Administration (FDA) and CDC anticipated the strains most likely to infect people this season. The vaccine contains antigens for influenza A (H1N1) [A/Beijing/262/95-like]; influenza A (H3N2) [A/Sydney/05/97-like]; and influenza B [B/Beijing/184/93-like]. Influenza A (H3N2) dominated the virus strains recovered by culture at MDCH from October through January. Currently, the occurrence of influenza B (B/Beijing/184/93-like) is the predominant strain isolated at MDCH. Nationally, approximately 75% is type A (H3N2) and 25% is type B.

Again this year, MDCH is participating in the CDC study designed to determine the cost effectiveness of vaccine use among healthy adults under age 65. MDCH was asked to continue this study for another year because of last season's poor match between the vaccine and the circulating influenza strain. Results of the study are expected this summer.

Enteric Typing News

Linda Reese
Enteric/STD/Chromatography Unit

This past year MDCH received some unusual isolates of *Salmonella* spp. and *Shigella* spp. Isolates that have not been identified previously at MDCH laboratory are forwarded to CDC for confirmation after identification. There are over 2,000 possible known serotypes of Salmonella. *Salmonella* serotype Magwa was confirmed to be an extremely rare serotype. CDC had not previously seen it in their laboratory and only had a stock strain from 1955. Two other serotypes were confirmed at CDC as being rare in this country. They were *Salmonella* serotype Berkeley and *Salmonella* serotype Uzaramo. Usually MDCH identifies several rare serotypes every year. The following is a list of uncommon serotypes identified at MDCH during the past year:

Salmonella serotype Aberdeen
Salmonella serotype Albany
Salmonella serotype Bonn
Salmonella serotype Chameleon
Salmonella serotype Coeln
Salmonella serotype Concord
Salmonella serotype Hvittingfoss
Salmonella serotype Idikan
Salmonella serotype Kiambu
Salmonella serotype Kintambo
Salmonella serotype Matadi
Salmonella serotype Orion
Salmonella serotype Oritamerin
Salmonella serotype Romanby
Salmonella serotype Rubislaw
Salmonella serotype Singapore
Salmonella serotype Tel-el-Kebir

The majority of *Salmonella* spp. isolates (approximately 63%) identified at MDCH in the past year are in the list of the five most common serotypes: *Salmonella* serotype Typhimurium (which includes Typhimurium O-5 negative), *Salmonella* serotype Enteritidis, *Salmonella* serotype Heidelberg, *Salmonella* serotype Agona and *Salmonella* serotype Oranienburg.

Besides identifying Salmonella serotypes, MDCH also types and groups *Shigella* spp. isolates. This past year several *Shigella flexneri* were submitted to CDC that reacted in polyvalent B antisera but not in any of the individual types. CDC confirmed this and stated that these may represent a new provisional serotype of *Shigella flexneri*. *Shigella boydii* 1, *Shigella boydii* 10, *Shigella dysenteriae* 2 and *Shigella dysenteriae* 4 are also rare types that have been typed at MDCH.

Recently, MDCH identified a non-motile strain of *Escherichia coli* O157 which ferments sorbitol within 24 hours and produces Shiga toxins 1 and 2. This is the first *E. coli* O157 strain with this combination of characteristics ever submitted to CDC.

(Ed. Serotyping *Salmonella* spp., *Shigella* spp. and *E. coli* O157:H7 is an important tool to detect outbreaks of enteric diseases. MDCH encourages clinical laboratories to submit isolates for typing. The microbiology laboratory would like to acknowledge North Ottawa Hospital in Holland, Michigan for the isolation of the O157 mentioned above.)

DFA Protocol for *Bordetella pertussis*

The MDCH Laboratory and CDC do not offer direct flourescent antibody testing of nasopharyngeal swab smears for Bordetella pertussis. The reagents currently available lack sufficient specificity to be reliably used as a diagnostic assay from clinical specimens. The laboratory is developing an amplified test for this purpose. Until it is available, please submit only nasopharyngeal swabs for culture when requesting Bordetella pertussis examination. Acute and convalescent paired sera may also be submitted for serologic testing.

Virology Director Retires

Louis Guskey, Ph.D., virology section director retired January 15, 1999 after 14 years of service. Guskey joined MDCH in 1984 as the assistant director of virology and was promoted to director in 1993. During his tenure, the MDCH virology laboratory has undergone many transitions including a move to a new laboratory facility, institution of HIV seroprevelance studies, initiation of Hepatitis C testing and establishment of hantavirus testing. Guskey received his doctorate and master's degrees in virology from Ohio State University. Prior to joining MDCH, Guskey was active in the research of various human viral pathogens and taught at the University of Wisconsin, Milwaukee. Please join the virology section in thanking Dr. Guskey for his many years of steady leadership and in wishing him a long and meaningful retirement.

Quirky Bugs... Listeria Outbreak

Thomas F. Edwards, B.S., M(ASCP)
Reference Bacteriology Unit

A multistate outbreak of listeriosis caused the recall of processed meat products from Michigan. The Chicago-based Sara Lee company recalled 300 types of meat products from its Bil Mar plant in Zeeland, Michigan. CDC has reported 70 cases since August 1998, including 12 deaths and three miscarriages traced to a single strain of *Listeria monocytogenes*. All isolates from these cases are serotype 4b, and share an unique pattern when subtyped by pulsed-field gel electrophoresis (PFGE).

The genus Listeria was first described in 1928 and for many years had only one species. Five other species have been added since 1948 with *Listeria monocytogenes* the only human pathogen. *L. monocytogenes* is a Gram positive, asporogenous, rod that is short, straight, and appears singly or in short chains. The cells are motile with 1-5 peritrichous flagella. This motility is demonstrated ideally in a semisolid agar medium incubated at 28°C. Colonies are small (1-2 mm), blue/gray, translucent and weakly beta hemolytic. Optimal temperature for growth is between 30° and 37°C. However, *L. monocytogens* is able to reproduce at temperatures as low as 4°C. Catalase is produced, oxidase is negative and dextrose is fermented. The Volges-Proskauer, methyl red, esculin hydrolysis and 6 1/2% salt tests are all positive.

L. monocytogenes is widely distributed in nature with many opportunities to enter food production. It can be found in soil, decaying vegetation, water, animal feed, fresh or processed meats, raw milk and cheese (especially the soft varieties), as well as asymptomatic human and animal carriers.

In adults, *L. monocytogenes* can cause septicemia, meningitis or encephalitis, primarily in the elderly or those patients with predisposing conditions that lower cell mediated immunity such as lymphoma, organ transplant or AIDS. It can cause a bacteremia in pregnant women that may also infect the fetus resulting in still birth or spontaneous abortion. On rare occasions persons without a known predisposing condition can become infected. Mortality is 20-50% if the CNS is affected. The mode of transmission is primarily through ingestion, although cutaneous listeriosis has been reported among veterinarians and abattoir workers.

MDCH has tested 16 *L. monocytogenes* isolates since October 8, 1998; 12 from patients (11 blood cultures, one from amniotic fluid) and four from food products. Suspected food products were received from the vendors before sale to the public and sent to the Michigan Department of Agriculture Laboratory for analysis. PFGE analysis shows nine distinct patterns distributed over the 16 isolates as follows:

Specimen Source	MDCH PFGE Pattern	No. Isolates
Blood culture	1A	6
Blood culture	1B	2
Blood culture	4, 5 & 9	1 each
Amniotic fluid	8	1
Hot Dog 1st lot	2	1
Hot Dog 2nd lot	3	1
Bologna	7	1
Sandwich Spread	6	1

Thus far, MDCH has been unable to isolate a *L. monocytogenes* from food consumed by ill persons. Results are pending on two of the PFGE 1A and the hot dog isolates sent to CDC to determine if they are outbreak associated. All clinical laboratories in Michigan are encouraged to submit any human *L. monocytogenes* isolates to MDCH for confirmation and PFGE analysis to establish any possible links to this outbreak.

(Ed.: Laboratory diagnosis of Listeriosis is made by culture of blood or sterile fluid. Stool culture is not helpful in diagnosis. Currently the Oscar Meyer company has found *L. monocytogenes* in two types of luncheon meat products resulting in a recall. The Southfield, Michigan-based Thorn Apple Valley company has recalled hot dogs and luncheon kits produced during a six-month period at its Forrest City, Arkansas plant for Listeria contamination. Bosell Foods, Inc. of Cleveland has also recalled sliced ham products distributed in the Cleveland area for this problem. For current recalls see the USDA food safety webpage at:

http://www.fsis.usda.gov/ophs/recalls/recalls.htm)

Congratulations !!!

Congratulations go to Barbara Robinson-Dunn. Ph.D., director of microbiology, who recently completed her third three-year term on the editorial board of the American Society for Microbiology's *Journal of Clinical Microbiology*. Robinson-Dunn hopes to return to the editorial board after a one-year hiatus.

REPLACEMENT OF MHA-TP WITH TP-PA TEST FOR SYPHILIS ANTIBODY CONFIRMATION

Steve S. Hsu, Ph. D.
Bacterial & Parasitic Serology Unit

The sexually transmitted disease syphilis, caused by the Gram negative spirochete *Treponema pallidum,* has been recognized for centuries as a devastating illness. It remains a public health concern worldwide. The organism is transmitted through direct contact of mucous membranes or abraded skin with an infectious lesion. It is both an acute and a chronic infection with many diverse clinical manifestations. It usually occurs in distinct stages that are separated by periods of latency or quiescence.

Depending on the stage of disease, quantitative and qualitative serological results vary. The most useful test to be performed depends the suspected stage of infection. Control and surveillance require careful screening tests by reliable methods. Serological testing should combine a non-treponemal screening test such as the Venereal Disease Research Laboratory (VDRL) test, the Unheated Serum Reagin (USR) test or the Rapid Plasma Reagin (RPR) test, and a treponemal confirmation test such as the Microhemagglutination *Treponema pallidum* (MHA-TP) test.

Results of non-treponemal tests usually parallel the status of infection. Titers are high during acute infection and then decrease during subclinical infection (latency) or after effective antibiotic therapy. In contrast, the treponemal test may not become reactive until after the initial acute manifestation. Titers may remain high in latency and after treatment. It is recommended that non-treponemal tests be used for diagnosis in early stages and for the evaluation of a cure after antibiotic therapy. The treponemal tests are used for diagnosis of latency. When the non-treponemal tests are used as a screening test, all reactive tests should be confirmed with a treponemal test. In low risk populations, up to half of all reactive non-treponemal tests may be false positive results.

The Centers for Disease Control and Prevention (CDC) issues guidelines to manufacturers and developers of syphilis tests for the evaluation and standardization of syphilis diagnostic reagents. Each new or revised test progresses through three stages of evaluation. In the investigational stage the test is evaluated in parallel with standard tests to define clinical and laboratory usefulness. During the provisional stage the test evaluated in large scale studies. Tests that perform acceptably in these evaluations are given provisional status and are acceptable for reporting patient results. In the standard stage of evaluation the specific written technique undergoes large scale evaluations to prove it is useful for medical management.

The three standard treponemal tests use *T. pallidum* as an antigen and are based on the detection of patient antibodies against bacterial cellular components. In the Fluorescent Treponemal Antibody Absorption (FTA-ABS) test the patient antibodies will bind to slide fixed antigen. Bound antibody-antigens are then visualized via fluorescence with anti-human IgG conjugate. The FTA-ABS is the only treponemal test designated by CDC as a standard status test. Two other treponemal tests are microhemagglustination procedures. These two differ mainly in the source of the erythrocytes used as carriers for the sonicate of *T. pallidum.* Sheep red blood cells are used in the MHA-TP and turkey blood cells in the Hemagglutination Treponemal Test for Syphilis (HATTS).

Previously, MDCH offered the MHA-TP procedure as a routine confirmatory treponemal test. The MHA-TP has been replaced by the *T. pallidum* Particle Agglutination (TP-PA) test (1). The TP-PA uses the same treponemal antigen as the MHA-TP test but also uses colored gelatin particles sensitized with *T. pallidum* antigen. The TP-PA test is reported to yield fewer nonspecific reactions and is more clearly interpreted in the laboratory. Although this test is approved by the Food and Drug Administration, the manufacturer has not sought its approval by CDC as a provisional or standard test.

Deguchi et. al. (2) evaluated 800 specimens from infected and healthy patients. The rate of agreement between the results of TP-PA and MHA-TP methods was 97.8%. The FTA-ABS method was used to further characterize the non-concordant specimens with results almost completely coinciding with the results obtained from the TP-PA method. Validation studies in our laboratory and at the CDC indicate that the TP-PA test is an acceptable alternative to the MHA-TP test.

There are several technical advantages to the laboratory for using the TP-PA procedure. It is less technically demanding than the MHA-TP. No pre-dilution or incubation of the sample are necessary. Serum without contamination is acceptable for testing. A sample volume of 2 to 5 ml is preferred for testing. The plastic skirt-capped tubes and the FB200 test request form are supplied in the MDCH Unit #8. Kits are available from the Specimen Container Order Unit (517) 335-9867. Questions regarding specimen submission or testing should be directed to Dr. Steve Hsu at (517) 335-8100.

Note: The Captia Syphilis EIA has CDC provisional status as a confirmatory test. The only EIA test with provisional status as a screening test is the Spirotek Reagin II EIA.

References:

1. Kit Package Insert for Serodia TP-PA Reagents for the Detection of Antibodies to *Treponema pallidum* Fujirebio Inc., Chuo-Ku, Tokyo 103, Japan. Distributed by Fujirebi America Inc., Fairfield, NJ 07004.

2. Deguchi, M., H. Hdsotsubo, N. Yamashita, T. Ohmine, S. Asri, "Evaluation of the Gelatin Particle Agglutination Method for Determining *Treponema pallidum* Antibody," Journal of the Japanese Association for Infectious Diseases 68:#10, 1994.

3. Larson, S., V. Pope, R.E. Johnson, E.J. Kennedy, Jr., A Manual of Tests for Syphilis, 9th Edition, American Public Health Association, Washington, DC, 1998.

Diagnostic Tests Evaluated
by the U.S. Public Health Services (CDC)

	Standard	Provisional	Investigational
Non-Treponemal	VDRL Unheated Serum Reagin (USR) Rapid Plasma Reagin (RPR) Toluidine Red Unheated Serum Test (TRUST)	Spirotek Reagin Visuwell Reagin	
Treponemal	Flourescent Treponemal Absorption (FTA-ABS) FTA-ABS Double Staining (FTA-ABS DS) MHA-TP* Dark Field Microscopy Direct Flourescent Antibody test for *T. pallidum* (DFA-TP)	Captia Syphilis-G	Spirotek Syphilis Test Visuwell Syphilis Test

*No longer commercially available

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ERRATA

In the Fall 1998 issue of the LabLink (Vol.4, No.2) the following errors were made in the column "Quirky Bugs... Challenging Respiratory Isolates from CF Patients":

1. *The credentials for Stephen Haskell should read "BS, M, SM (ASCP).*
2. *The fourth paragraph should read "Typical B. cepacia is described as a Gram negative straight or slightly curved rod; motile (polar tuft of three or more flagella); oxidizing glucose, xylose, etc.*

Also, the correct address for the Bureau of Laboratories webpage is:

www.mdch.state.mi.us/pha/bofl

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